II. RESPONSE TO OFFICE ACTION

A. Status of the Claims

Claims 1-47 were pending at the time of the Office Action dated May 14, 2004. Claims 12, 24 and 43 have been amended and 48 added herein. The Commissioner is authorized to deduct any additional claim fees that may be due from Fulbright & Jaworski L.L.P. Account No.: 50-1212/UTSB:715US. Support for claim 48 is found in claims 1, 2, 6 and page 11, lines 1-6. No new matter is added by the amendments.

Claims 1-48 are now pending and presented for reconsideration.

B. Status of the Specification

The specification has been amended at page 18, lines 1-8 to correct a typographical error. The specification inadvertently recited "arginine," instead of the correct amino acid, "aspartate." Support for the amendment can be found in the specification on page 17, lines 23-26, where it is correctly explained that aspartate, and not arginine, directs a protein to remain anchored to the inner membrane, as is known in the art. No new matter has been added by the amendment.

C. Claim Objection

The Action objects to claim 43 for reciting "FACS," instead of the full term for which this stands, "Fluorescence Activated Cell Sorting." In response, Applicants note that the recited term has been amended to include the full name for the term. The amendment does not change the scope of the claim and thus Applicants do not intend to disclaim any subject matter through the amendment. Removal of the objection is respectfully requested in view of the foregoing.

D. Rejection of Claims Under 35 U.S.C. § 112, Second Paragraph

The Action rejects claims 1, 3, 12, 24 and 43 under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out the subject matter which Applicant regards as the invention. In particular, the Action alleges (1) that the meaning of the term "capable of" cannot be ascertained in claims 1, 3, and 24, (2) that the meaning of the term "under conditions" cannot be ascertained in claims 1 and 43, and (3) that the meaning of the term "obtained by" cannot be ascertained in claim 12.

(1) The term "capable of"

The term "capable" as used in the claims is fully definite because it has a well known meaning in the art. The term "capable of" is understood to those of skill in the art to mean having the ability to complete a specified task or function. For example, the relevant definition of the term "capable" from the online version of the Encarta™ dictionary (http://encarta.msn.com/dictionary_/capable.html) is given as "able to do particular thing: possessing the qualities needed to do a particular thing." **Appendix A**. The relevant definition from the online version of the Merriam-Webster's Collegiate Dictionary (http://www.m-w.com/dictionary.htm) is given as "having attributes (as physical or mental power) required for performance or accomplishment." **Appendix B**.

Claim 1 refers to a labeled ligand that is "capable of contacting the binding polypeptide." Given the well known meaning of the term "capable" and the derivative "capable of," the meaning of these terms as they are used in the claim is definite. Thus a labeled ligand "capable of contacting the binding polypeptide" refers to a labeled ligand having the ability to contact a binding polypeptide. The use of "capable of" in claim 1 is thus not indefinite, because "capable of" is a readily ascertainable standard well known to those of skill in the art.

Claim 3 refers to a leader sequence that is "capable of directing the expression of said fusion polypeptide to the outer side of the inner membrane." This term is not indefinite, because whether a leader sequence has the ability to direct the expression of a fusion polypeptide to the outer side of the inner membrane is readily ascertainable and understood by one of skill in the art. A variety of such leader sequences are known in the art and described in the specification. The unifying characteristic of the leader sequences is that in a cell they act to, e.g., are capable of, directing an expressed polypeptide to the outer side of the inner membrane. Indeed, this is illustrated in the working examples. The use of "capable of" in the claim 3 is thus not indefinite, because the term represents a readily ascertainable standard well known to those of skill in the art.

Claim 24 refers to a nucleic acid that is "capable of being amplified." This term is not indefinite, because whether a nucleic acid has the ability of being amplified is readily ascertainable and understood by one of skill in the art. Applicants nonetheless note that, in the interest of compact prosecution of the case, the claim has been amended. The claim does not narrow the scope of the claim and thus Applicants do not intend to disclaim any subject matter through the amendment. It is believed that the rejection of claim 24 for indefiniteness is now moot.

The second paragraph of 35 U.S.C. §112 requires only that it be clear to those skilled in the art what Applicant intends to claim. What is dispositive is whether one of ordinary skill in the art would understand what is claimed when the claims are read in light of the specification. Where, as here, one of skill would readily understand the meaning of a given term, the use of that term in the claims is not indefinite as set forth above. Removal of the rejection is thus respectfully requested.

(2) The term "under conditions"

Claims 1 and 43 refer to "contacting the bacterium with a labeled ligand under conditions wherein the labeled ligand is capable of contacting the binding polypeptide." The use of this term is not indefinite because both the claims and specification fully describe those conditions. For example, page 42, lines 12- 18, provide a detailed description of conditions wherein a labeled ligand contacts a binding polypeptide. This, together with the knowledge of those of skill in the art, fully allows one skilled in the art to readily ascertain the specific conditions under which a bacterium can be labeled. Because the specification clearly defines conditions under which a labeled ligand can contact the binding polypeptide, the term "under conditions" in claims 1 and 43 is not indefinite.

(3) The term "obtained by"

Claim 12 refers to a population of bacteria "obtained by a method." Although Applicants assert that the claim as originally written was not indefinite, Applicants draw the examiner's attention to the amendment to claim 12. Claim 12 has been amended to replace "obtained" with "produced." The claim does not narrow the scope of the claim and thus Applicants do not intend to disclaim any subject matter through the amendment. Applicants believe that in view of the amendment, the rejection of claim 12 for indefiniteness is now moot.

In light of the foregoing, Applicants hereby respectfully request that the rejection of claims 1, 3, 12, 24 and 43 under 35 U.S.C. §112, second paragraph, be withdrawn.

E. Rejection of Claims Under 35 U.S.C. § 112, First Paragraph

The Action rejects claims 1-47 under 35 U.S.C. § 112, first paragraph, as lacking enablement. In particular, the action asserts four points of non-enablement: (1) that the use of

fragments of transmembrane proteins or inner membrane lipoproteins is not enabled, (2) that a wash step is necessary to practice the claimed methods, (3) that the specification does not enable contacting a bacterium with any labeled ligand, and (4) that the specification does not enable contacting a bacterium with a labeled ligand comprising a nucleic acid molecule. Applicants traverse these rejections as set forth below.

(1) The limitations of the dependent claims were not addressed.

Before addressing the individual rejections, Applicants first respectfully note that the rejections have not been applied with respect to the claimed invention as recited in each claim. For example, the Action rejects all of the claims for lacking a wash step. However, such a step, although demonstrated below by declaratory evidence to be unnecessary for enablement, is specifically recited in claim 34. This claim reads as follows: "[t]he method of claim 1, further comprising removing labeled ligand not bound to said candidate binding polypeptide." Similarly, the Action rejects all of the claims as not being enabled for use of ligands of greater than 2000 Da on the basis that such ligands allegedly could not cross the outer membrane. Again, although the evidence presented below shows this to be incorrect, claim 31 already recites "[t]he method of claim 1, further comprising removing the outer membrane of said bacterium." Thus any argument that use of all labeled ligands is not enabled because they could not cross the outer membrane is irrelevant and should not be made with respect to this claim.

Therefore, in the event that any of the current rejections are maintained, Applicants respectfully request that the limitations of each independent and dependent claim be fully and individually addressed. This is required for compliance with the repeated holdings of the Federal Circuit finding that enablement must be applied with respect to the claimed invention. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). To ensure consideration of specific commercial

embodiments that have yet to be fully addressed in the Action, Applicants have therefore added new claim 48.

(2) Applicants have enabled anchoring using protein fragments

The Action, while acknowledging that the specification is enabling for binding proteins anchored using transmembrane proteins or inner membrane lipoproteins, states that the use of fragments of transmembrane proteins or inner membrane lipoproteins is not enabled. Applicants traverse, as the working examples show use of precisely such fragments.

Examples 1-4 show that anchoring was achieved by use of a fragment comprising the first six amino acids from new lipoprotein A (NlpA). The working examples therefore directly show enablement for anchoring of a binding protein to the inner membrane using a fragment from an inner membrane protein. In further support of this, Applicants have submitted as **Appendix C** the declaration of inventor Barrett Harvey. As described by Dr. Harvey, changing the second amino acid of a lipoprotein or lipoprotein fragment to an Aspartate residue will target the protein to the inner membrane (Harvey Declaration, page 3 paragraph 6-A). This is described in the specification at page 17 lines 23-26 and the technique is demonstrated in Examples 1-4 of the specification, where the method of claims was practiced by anchoring a single chain antibody to a fragment from NlpA. Dr. Harvey explains that because any lipoprotein fragment can be targeted to the inner membrane, Examples 1-4 show that any lipoprotein fragment can be used to practice the method of the claims.

Further, Example 15 shows that anchoring was also achieved by use of a short variant of the phage gene III minor coat protein (g3p). The specification therefore enables a person skilled in the art to anchor a binding protein to any fragment of a transmembrane protein. As described by Dr. Harvey, during normal phage morphogenesis, g3p resides across the inner membrane

before it is incorporated into a growing phage (see specification page 67 lines 30-31 and Harvey Declaration, page 3, paragraph 6-B). Expression of the short variant of g3p without the other genes of the phage causes the g3p variant to remain attached to the inner membrane as a transmembrane protein. Dr. Harvey explains that because the short variant of g3p is a fragment of g3p, Example 15 shows that a fragment of a transmembrane protein can be used to practice the method of the claims.

As is further described by Dr. Harvey, subsequent studies have shown use of the method described in claim 1 using a wide variety of fragments of the *E. Coli* transmembrane protein TatC (see Harvey Declaration, page 4 paragraph 7-B). It was demonstrated in particular that at least 28 different fragments from TatC can be successfully used to anchor a binding polypeptide to the periplasmic side of the inner membrane. The figure attached as Exhibit A to **Appendix C** illustrates the location and identity of the 28 different fusion points between the TatC fragments and the binding protein. This figure demonstrates that, in the clones selected using the method described in the application, the binding protein was fused to sites that were periplasmic or were near the periplasmic end of a transmembrane helix. As Dr. Harvey concludes, the specification enables those of skill in the art to create and screen a library of fragments from essentially any transmembrane gene, so that the method of claim 1 can be achieved using fragments from essentially any transmembrane protein without undue experimentation.

The foregoing and workings examples therefore demonstrate that a broad range of anchors can be used to practice the method of the invention, and that the specification fully enables this subject matter. A specification that "contains **a** teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented **must** be taken as being in compliance with

the enablement requirement." *MPEP* § 2164.04 (2003) (emphasis added). As Applicants have demonstrated that the specification enables one with skill in the art to anchor a binding protein using a fragment from an the inner membrane protein or a fragment from a transmembrane protein, it is respectfully submitted that the rejection has been overcome and removal thereof is respectfully requested.

(3) Applicants have demonstrated that a wash step is not necessary

The Action alleges that a wash step is necessary to practice the claimed invention. It is stated that, since labeled ligand could be present in a bacteria yet not be bound, one practicing the claimed method without a wash step could not distinguish between cells containing bound ligand and cells contained unbound ligand.

In response, it is noted that the addition of a wash step is not required for use of the invention as alleged in the Action. In support of this, Applicants have provided a copy of the Declaration of Jongsik Gam (attached as **Appendix D**), which was submitted in Application Serial No. 09/699,023, a parent application of this case. As described by Mr. Gam, the following technique was successfully carried out without a wash step:

> A method of obtaining a bacterium comprising a nucleic acid sequence encoding a binding protein capable of binding a target ligand comprising the steps of:
>
> (a) providing a Gram negative bacterium comprising a nucleic acid sequence encoding a candidate binding protein, wherein said binding protein is expressed in soluble form in the periplasm of said bacterium;
>
> (b) contacting said bacterium with a labeled ligand capable of diffusing into said periplasm; and
>
> (c) selecting said bacterium based on the presence of said labeled ligand within the periplasm, wherein said ligand and said candidate binding protein are bound in said bacterium.

As described by Mr. Gam, the claimed methods of the parent application have been used both with and without the addition of a wash step to demonstrate that the wash step is not necessary. It was demonstrated in particular that cells expressing a binding protein having affinity for a labeled ligand can be detected away from control cells based on the presence of the labeled ligand bound in the periplasm with or without a wash step. Mr. Gam explains that the specific interaction of the binding protein and labeled ligand in the periplasm of the bacterium ***retains*** and ***concentrates*** the labeled ligand inside the periplasm of ***only*** those cells with high affinity binding proteins. Thus in the methods of the parent application, the concentration of the labeled ligand bound to the binding protein in the periplasm makes the cells detectable regardless of the presence of unbound labeled ligand.

In the method of claim 1 of this application, the interaction between the binding protein and labeled ligand also retains and concentrates the labeled ligand in only those cells with high affinity binding proteins. The method of claim 1 concentrates labeled ligand based on the same binding principles as the methods of the parent application, and also makes cells detectable regardless of the presence of unbound labeled ligand. Therefore, like the claimed methods of the parent application, the method of claim 1 can be achieved with or without a wash step.

As Applicants have demonstrated that this additional step is not required, and the claimed method is fully enabled without the step, removal of the rejection is respectfully requested.

(4) Applicants have enabled contacting a bacterium with any labeled ligand

The Action contends that because only certain ligands can cross the outer membrane of a gram negative cell, the specification does not enable contacting a bacterium with any labeled ligand. It argues in particular that the specification does not teach the use of labeled ligands having a molecular weight of 2000 Da, and therefore fails to enable the use of all labeled ligands.

In support of this it is stated that bacterial cells comprise certain transport mechanisms that limit both the size and type of molecules able to cross the bacterial outer membrane.

As an initial matter, Applicants note that claim 1 already specifically requires that the bacterium be contacted "under conditions wherein the labeled ligand is capable of contacting the binding polypeptide." Therefore, the situations envisaged in the Action in which a given ligand does not have access to the binding polypeptide in the periplasm are specifically ***excluded*** from the claims. Enablement is viewed from the perspective of the ***claimed*** invention. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). What is relevant is that Applicants have described such conditions under which the ligand has access to the binding protein, not unclaimed eventualities.

Enablement speaks to the ability to make and use the claimed invention. The mechanisms by which ligands enter the cell is irrelevant to the function of the technique. In this regard, it is initially noted that the references cited as teaching that molecules of only up to 900-2000 Da can enter the cell or by specific transporter systems did not use conditions to permeabilize the gram negative bacteria. In contrast, the specification teaches numerous treatments in an entire section devoted to methods of permabilizing the outer membrane, including use of polymyxin B nonapeptide, OmpF overexpression, hyperosmotic shock, calcium ions, Tris buffer, filamentous phage inner membrane protein pIII, multimeric outer membrane protein pIV, and combinations thereof (see p. 15-16 of the specification). These treatments do not require any particular transporter system and thus are non-specific.

For instance, Example 9 of the specification demonstrates increased permability in bacterial cells grown at sub-optimal temperature. In FIG. 8 of parent application Serial No. 09/699,023, an increase in FACS signal was obtained when cells expressing periplasmic scFv antibodies to digoxigenin were labeled with this probe using 5X PBS to permeabilize the outer

membrane. The probe was greater than 2000 Da and thus the assertion of a lack of enablement is incorrect.

Applicants further note that additional studies have also demonstrated the enablement of the claims with even larger labeled ligands. Applicants direct the Examiner's attention in this regard to the paper by Chen *et al.*, (*Nature Biotechnology*, 2001, 19, 537-542; submitted as IDS ref. C5). There shown are studies demonstrating that oligonucleotide 10mers (4,897 Da) and even 20mers (8,727 Da) labeled with digoxigenin and a fluorescent label can successfully label *E. coli* in 5X PBS expressing scFv antibodies to digoxigenin in the periplasm. (see Fig. 3, page 539).

Further, studies carried out under the supervision of the inventors have also demonstrated that appreciable labeling was obtained using a peptide construct of molecular weight 2,505 Da or a polyethylene glycol conjugate of molecular weight 2,937 Da. In both cases the conjugates contained digoxigenin attached to one end and a fluorescent label on the other. Specific labeling was only seen with *E. coli* cells expressing the 26-10 antidigoxin antibody scFv in the periplasm. The results were achieved using polymyxin B nonapeptide (PMBN) as the outer membrane permeabilizer (specification, page 15).

What the foregoing studies and the specification demonstrate is that labeling of *E. coli* cells has been achieved using three entirely different classes of molecules (oligonucleotides, oligopeptides and a polyethylene glycol derivative), with molecular weights between 2,000 Da and almost 9,000 Da, by adding reagents that partially disrupt the outer membrane. These permeabilization strategies, including 5X PBS and PMBN, act to ***destabilize the outer membrane*** as opposed to triggering any specific transport mechanism. The technique is therefore not specific to any class of molecule. The selectivity or lack thereof of any given cell

membrane transport system is therefore irrelevant to the function of the claimed method. Knowledge of the exact identity of any transport systems involved with entry of larger ligands into the cell is also not required for use of the invention. As such, there is no basis to conclude that the invention is limited to any given class of labeled ligand.

The same type of techniques for permeabilizing the outer membrane described in this application are described in Chen *et al.*, such as treatments with filamentous bacteriophages or growth under sub-optimal conditions. That is, the specification ***specifically teaches*** these techniques as well as many other such techniques for permeabilization of bacteria. For example, the use of ***filamentous bacteriophages*** to increase permeability is described in the last paragraph of ***page 16*** of the specification. The use of ***sub-optimal temperatures*** is specifically taught in the first paragraph of ***Example 3*** of the specification. The specification further describes numerous other techniques that were known in the art for increasing bacterial permeability. For example, Fig. 8 shows that an increase in FACS signal was obtained when cells expressing periplasmic scFv antibodies to digoxigenin were labeled with this probe using 5X PBS to permeabilize the outer membrane. Therefore, given that the claims require that the ligand be used under conditions in which it is capable of contacting the binding polypeptide in the periplasm and the conditions for doing so are fully described in the specification, there is no basis to conclude that Applicants' claims are not fully enabled.

(5) The use of nucleic acids has been enabled

The Action contends that hydrophilic nucleic acid molecules cannot cross the hydrophobic outer membrane of a bacterium, and the specification is thus not enabling for a labeled ligand comprising a nucleic acid molecule. The Action further contends that since a labeled nucleic acid could hybridize to any nucleic acid molecule already present in the bacterial

cell, one skilled in the art could not distinguish between cells containing ligand hybridized to the nucleic acid molecules of the bacteria, and cells containing ligand bound to the candidate binding protein.

In response, Applicants note that the issue appears to be moot in light of the comments above demonstrating that the method of the invention are limited to any particular type or size of ligand. Applicants also direct the Examiner's attention in this regard to the paper by Chen *et al.*, (*Nature Biotechnology*, 2001, 19, 537-542), provided here in **Appendix E**. There shown are studies demonstrating the use of labeled ligands comprising nucleic acids for detection in the periplasm of *E. coli* bacteria expressing a binding protein. In particular, oligonucleotide 10mers (4,897 Da) and even 20mers (8,727 Da) were labeled with digoxigenin and a fluorescent label, resulting in the successful labeling of *E. coli* in 5X PBS expressing scFv antibodies binding the digoxin in the periplasm. (see Fig. 3, page 539). The studies demonstrate enablement for use of nucleic acids.

Use of labeled ligands comprising nucleic acids is also described in Example 8 of Application Serial No. 09/699,023, which is the parent application of this case. This example demonstrates that a fluorescently-tagged oligonucleotide (2384 Da molecular weight) diffused into bacteria, and specific ligand-binding protein binding occurred. In addition, no hybridization was observed between the labeled nucleic acid and nucleic acid molecules already present in the bacterial cell. Because no evidence exists that this kind of interaction would present a significant problem for use of the invention, the idea that hybridization will prevent use of the invention must be regarded as speculation. The nucleic acids that are used as ligands can be easily distinguished from other nucleic acids because the ligand nucleic acids are tagged with a fluorescent (or other) labeling moiety.

The studies above demonstrate that nucleic acids can enter the periplasm. They further demonstrate that the entry of labeled ligands into the periplasm is not limited to only certain types of molecules of a limited size. In this method of the invention, the nucleic acid is not used as a source of genetic information to be transcribed and translated into protein. Rather, the nucleic acid is being used as a scaffold to contain the ligand of the binding protein as well as a fluorescent label. Therefore, again, non-specific binding with native nucleic acids does not affect the ability to identify selectively bound labeled ligand.

As applicants have demonstrated that the use of a ligand that can cross the outer membrane by any particular mechanism is not required for use of the invention, and the claimed method is fully enabled for labeled ligands comprising nucleic acid molecules, removal of the rejection is respectfully requested.

In view of the foregoing, removal of the rejections under 35 U.S.C. §112, first paragraph, is respectfully requested.

F. Conclusion

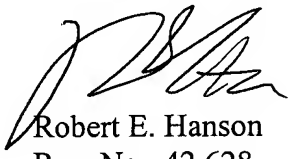
In light of the foregoing, applicants submit that all claims are in condition for allowance, and an early indication to that effect is earnestly solicited. The examiner is invited to contact the undersigned (512)536-3085 with any questions, comments or suggestions relating to the referenced patent application.

III. PETITION FOR EXTENSION OF TIME

Pursuant to 37 C.F.R. § 1.136(a), Applicants petition for an extension of time of one month to and including September 14, 2004 in which to file the instant response. Pursuant to 37 C.F.R. § 1.17, a check in the amount of $55.00 is enclosed, which the process fee for a one

month extension of time. If the check is inadvertently omitted, or should any additional fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to the instant response, or should an overpayment be included herein, the Commissioner is authorized to deduct or credit said fees from or to Fulbright & Jaworski L.L.P. Account No.: 50-1212/UTXB:715US.

Respectfully submitted,



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